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Accepted for publication 13 June 2002

Four layer discontinuous gradient for HIV

Artificial insemination using processed semen is a risk reduction option, if they want children, for serodiscordant couples in whom the man is HIV positive. The main aim of this study was to develop a single semen processing technique to reduce HIV transmission risks to HIV negative wives without infection and to obtain better quality sperm.

Methods

After ethics committee approval and written informed consent, normozoospermic semen was provided by two asymptomatic HIV carriers. Discontinuous four layer density gradient, whose fractions (Fr) were 1.065 (Fr 4), 1.085 (Fr 3), 1.110 (Fr 2), and 1.135 (Fr 1), was prepared with Puresperm. Semen washed with Hank's solution was laid on this gradient and centrifuged at 400 g for 30 minutes. The specimen of each fraction was extracted to determine sperm quality and to detect HIV RNA and proviral DNA using RT-PCR and PCR, respectively. Lymphocytes of an HIV non-carrier were co-cultured for 4 weeks with each fraction. HIV p24 antigen and proviral DNA after co-cultivation with each fraction were determined by indirect immunofluorescence assay and polymerase chain reaction (PCR), respectively.

Results

The percentage collection of sperm from Fr 1, Fr 2, Fr 3, and Fr 4 was 3% (SD 2%), 32% (9%), 19% (8%), and 10% (4%), respectively. Motility rate was 55% (19%), 94% (4%), 57% (25%), and 19% (11%), respectively. HIV proviral DNA and HIV RNA were detected only from Fr 4. HIV p24 antigen was observed in the lymphocytes co-cultivated with Fr 4 and from the positive control, but was not observed in other fractions. HIV proviral DNA was not detected from Fr 2 or Fr 3 (tables 1 and 2).

Table 2 Detection of HIV p24 antigen and proviral DNA after 4 weeks' co-cultivation with each fraction and carrier's PBL

	HIV p24 antigen	HIV DNA
Fr 1	neg	pos
Fr 2	neg	neg
Fr 3	neg	neg
Fr 4	pos	pos
Carrier's PBL	pos	pos

PBL = peripheral blood lymphocytes.

Discussion

HIV discordant couples have a risk of transmission generally if they wish to have a baby.¹⁻² Semprini *et al*³ reported continuous gradient centrifugation followed by a swim up procedure, and Marina *et al*⁴ carried out a similar method but HIV was detected in 5.6% of 107 samples. However, the condition of the sperm, after these processes, was not always sufficient for intrauterine insemination.

We have developed a novel semen single processing technique to reduce HIV RNA and HIV proviral DNA to undetectable levels in the fraction whose sperm quality was higher than others. Furthermore, this fraction was confirmed to have no HIV infectivity in vitro. This method appears to be an attractive alternative for HIV discordant couples.

Contributors

KK and YA contributed to laboratory work; AY referred HIV positive volunteers.

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Accepted for publication 1 May 2002

Table 1 Sperm characteristics and detection of HIV in each fraction

	Sperm collection rate (%)	Sperm motility rate (%)	HIV RNA	HIV DNA	HIV p24 antigen after co-cultivation	HIV DNA after co-cultivation
Fr 1	3 (2)	55 (19)	negative	negative	negative	positive
Fr 2	32 (9)	94 (4)	negative	negative	negative	negative
Fr 3	19 (8)	57 (25)	negative	negative	negative	negative
Fr 4	10 (4)	19 (11)	positive	positive	positive	positive

Erythema nodosum induced by chancroid

Erythema nodosum is a type of panniculitis which is often regarded as a complex reaction pattern to various aetiological factors of infective and non-infective origin.¹ Infective agents outnumber inflammatory causes and drugs in causation of erythema nodosum in the developing countries. Almost all the infective agents including aerobic and anaerobic bacteria, viruses, fungi, parasites and mycobacteria can induce eruption of erythema nodosum.² Among sexually transmitted infections lymphogranuloma venereum has been known to be associated with erythema nodosum not infrequently.³

A 23 year old woman presented with genital ulcer disease and painful rash over the legs of 1 week's duration. There was no history of trauma, fever, or drug intake. She had a single stable sexual partner who was apparently unaffected. Examination revealed a single, 1-1.5 cm size, irregular tender ulcer on the right labia minora with undermined margins and bleeding on touch. The right inguinal lymph nodes were firm, moderately enlarged, and tender. Speculum and vaginal examination was normal. Examination of the perianal region, perineum, and other mucosae was also normal.

Multiple tender, erythematous nodular subcutaneous lesions with dusky erythema were present over both shins, calves, and ankle joints. Investigations revealed a normal complete blood count, serum biochemistry, urinalysis and blood sugar. VDRL, HIV-1 ELISA, and HBsAg were negative. Dark ground illumination, smears, and cultures from the ulcer did not reveal aetiological diagnosis. Histopathology from the ulcer revealed an ulcerated surface with necrosis and neutrophilic infiltrate deeper to which a zone of new blood vessel formation with marked endothelial proliferation and a lymphoplasmacytic infiltrate was observed. These features were consistent with diagnosis of chancroid while histopathology of leg lesions confirmed it to be septal panniculitis consistent with a diagnosis of erythema nodosum. The patient was treated with erythromycin stearate 500 mg 6 hourly for 7 days. The genital ulcer healed completely in 7-10 days but the lesions of erythema nodosum subsided completely in 5-7 days without any other treatment.

Erythema nodosum as a cutaneous reaction pattern was first observed by Willan in 1798.⁴ A female preponderance with a ratio of 3:1 is often observed in adults compared to an equal incidence at prepubertal age. Although the exact pathogenesis of erythema nodosum is not known, it has been regarded as an immune complex, deposition disease which prefers the richly supplied vascular adipose tissue of the legs.

In the present patient erythema nodosum and chancroid had a strong temporal correlation as erythema nodosum immediately followed the appearance of the chancroid and resolved completely with its resolution. Although erythema nodosum is known to be associated with innumerable infective agents, to the best of our knowledge chancroid leading to causation of erythema nodosum has not been observed before.

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Accepted for publication 5 June 2002

Gonococcal perianal abscess: re-emergence after cessation of co-trimoxazole

We report a case of perianal abscess due to *Neisseria gonorrhoeae*, which appears to have been suppressed but not eradicated by chronic low dose co-trimoxazole for a period of almost 6 months between acquisition and diagnosis.

The patient was a 34 year old HIV infected homosexual man treated with didanosine, stavudine, and nevirapine with a HIV viral load of 500 copies per ml and a CD4 lymphocyte count of $280 \times 10^6/l$. He was taking co-trimoxazole 400 mg/80 mg once daily to prevent *Pneumocystis carinii* pneumonia (PCP).

He reported last having receptive anal sex in June 2000. This was unprotected, with a casual partner at a "gay" sauna. Three weeks later he reported a perianal abscess which discharged spontaneously, requiring dressings for a few days. A sinus was observed and he was booked for elective surgery. He remained well for 5 months.

Co-trimoxazole PCP prophylaxis was stopped in November 2000 as his CD4 T lymphocyte count had remained above 200. Two weeks later (and almost 6 months after the last reported anal sex) he presented with purulent discharge emerging from a sinus approximately 3 cm from the anus.

N. gonorrhoeae (sensitive to penicillin, ceftriaxone, and ciprofloxacin) and *Bacteroides* species were cultured from this discharge. Swabs from the rectum, throat, and urethra as well as urine were negative for *N. gonorrhoeae* and *Chlamydia trachomatis* by polymerase chain reaction (PCR).

Oral ciprofloxacin was started but pain, swelling, and perianal cellulitis led to his admission to hospital where he was treated with intravenous ceftriaxone and metronidazole and surgical drainage.

Gonococcal perianal abscesses were reported in the pre-antibiotic era¹ but have disappeared from contemporary descriptions of gonorrhoea, whereas Bartholin's, periurethral, and tubo-ovarian gonococcal abscesses are described.²

The isolation of *Bacteroides* species and the worsening of the infection despite ciprofloxacin suggest that anaerobic organisms probably played a part in the development of an abscess, consistent with animal inoculation experiments.³ Another possible factor was the moderate immunosuppression (CD4 count of 280) from his HIV infection.

Six months passed from the time of infection to diagnosis, during which the patient was largely free of symptoms which then developed when co-trimoxazole was stopped. The likely explanation is that the

co-trimoxazole was suppressing the gonococcal infection without curing it. The failure to detect *N. gonorrhoeae* by PCR from the rectal specimen raises the possibility that co-trimoxazole may have eradicated a rectal infection in this case while only suppressing an extragenital manifestation.

It is now standard practice to stop PCP prophylaxis when CD4 counts rise above $200 \times 10^6/l$ in patients taking antiretroviral therapy.⁴ This may in turn have some impact on both the transmission and the manifestations of gonorrhoea in these patients, perhaps even contributing to increases in gonorrhoea in HIV infected populations.⁵

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Accepted for publication 14 June 2002

Uptake of HIV testing in patients with a confirmed sexually transmitted infection

UK seroprevalence rates indicate that up to 50% of HIV positive patients in genitourinary medicine (GUM) clinics remain undiagnosed.¹ HIV is mainly identified in high risk patient groups. Sexually transmitted infections other than HIV (STIs) have been shown to facilitate and be associated with enhanced HIV transmission.² Risk assessment for HIV, therefore, should target patients with an STI or history of recurrent STIs as a high risk group.

Targeting these patients to test for HIV at the time or 3 months after their STI

diagnosis, is important as it will lengthen the "diagnosis interval" of patients testing HIV positive thereby conferring a better outcome, with respect to HAART; identify patients with recent concurrent acquisition of HIV and a STI, entering a highly infective seroconversion phase; identify individuals with undiagnosed, established HIV infection and a newly acquired STI which promotes higher infectivity due to increased HIV viral shedding into genital secretions.^{4,5}

Our study analysed the uptake of HIV testing among attendees who had a genitourinary screen at St Thomas's Hospital genitourinary medicine department between 1 and 31 December 1999.

It compared the uptake of HIV testing, either at the index visit in December or deferred to within the ensuing 3 months, between patients diagnosed with an STI (gonorrhoea, chlamydia, herpes simplex virus, and trichomoniasis (study group)) and patients receiving a negative STI screen (control group).

Of 318 attendees, 242 and 76 patients comprised the study and control groups respectively. Only 18% (59/318) of patients tested for HIV on the initial visit. Significantly fewer of the study group tested for HIV (14%) compared to the control group (33%) ($p < 0.01$).

Of those who did not test for HIV, 11 and one patients deferred testing in the study and control groups respectively (table 1). However, none of the deferrers or initial non-testers re-attended for HIV testing in the following 3 months.

In view of this unacceptably low rate of HIV testing, both overall and in those patients with a confirmed STI, the following interventions are now being introduced, aiming to improve these figures and comply with the sexual health strategy 2001 targets.⁶

- An "opt out" policy of HIV testing
- Additional waiting room posters and a new patient information leaflet about HIV is given to all patients at registration to read while they wait to be seen explaining the natural history, treatments available, benefits of early diagnosis, and mechanisms of reducing transmission. This enhances patient education and may expedite consultation length and waiting times for patients with restricted "time off" and/or other more pertinent issues to discuss
- Pretest counselling is reserved for high risk groups instead of being required routinely
- Patients are able to obtain their HIV results indirectly, without the inconvenience of a previously required second visit
- Educating all GUM staff to encourage a high offer rate of HIV testing to all patients, especially targeting high risk patients, which incorporates those with a confirmed STI.

Table 1 Timeliness of HIV testing

	Tested for HIV at time of attendance	Deferred at time of attendance	Attended within 3 months and tested for HIV
Study	34/242 (14%)	11/242 (5%)	2/46 (4%)
Control	25/76 (33%)	1/76 (1%)	2/11 (18%)